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Thelephora iqbalii sp. nov. from the Himalayan moist temperate forests of Pakistan

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Abstract—A new thelephoroid species, *Thelephora iqbalii*, collected from the Himalayan moist temperate (HMT) forests of Pakistan, is characterized by small coralloid basidiomata bearing long, narrow ridges with fine hairs on their branches and hymenia that are cyanescent in KOH. An ITS-based phylogeny comparing *T. iqbalii* with other *Thelephora* species is also presented.

Key words—*Basidiomycota,* DNA, *Thelephoraceae*, angular lobate basidiospores

Introduction

The genus *Thelephora* Ehrh. ex Willd. is cosmopolitan (Corner 1968; Stalpers 1993; Kõljalg 1996; Maas Geesteranus 1971, 1975) and represented by 52 species worldwide (Kirk et al. 2008, Ramírez-López et al. 2017, Vizzini et al. 2016). Its basidiomata are distinguished by their pileate and stipitate/sessile or sometimes resupinate forms (Ahmad 1972).

Thelephora basidiomata range in shape from coral-like to distinctly pileate (Corner 1968). However, morphology alone is not always sufficient for correct identification. Recent molecular analyses have contributed to a better understanding of basidiomycete diversity (Gardes & Bruns 1993) and ITS-nrDNA primers are widely being used for this purpose (EI Karkouri et al. 2005, 2006; Gardes & Bruns 1993; Horton 2002).

The Himalayan moist temperate forests (HMT) have been previously included among 25 biodiversity hotspots in Indo-Burma (Myers et al. 2000), from which they were separated and designated as independent among the 34 currently recognized hotspots of biodiversity (Mittermeier et al. 2011).

Eight *Thelephora* species have previously been reported from Pakistan: *T. anthocephala* (Bull.) Fr., *T. arbuscula* Corner, *T. caryophyllea* (Schaeff.) Pers., *T. fucoides* Corner, *T. palmata* (Scop.) Fr., *T. penicillata* (Pers.) Fr., and *T. terrestris* Ehrh. (Ahmad 1956, 1972; Ahmad et al. 1997). Below we describe a new thelephoroid species from the HMT forests of Pakistan based on morphological and molecular evidence.

Materials & methods

Table 1. *Thelephora* rDNA sequences used in phylogenetic analyses

Taxon	Accession No.	Voucher No	Country
Thelephora americana	AY219838.1	UAMH 9578	Canada
Thelephora anthocephala	DQ974771.1	src614	USA
	AF272927.1	TAA165304	Estonia
Thelephora caryophyllea	GU234018.1	O75453	Netherlands
	AJ889980.1	TL-6566	Denmark
	EU326158.1	105	Austria
	EF655705.1	IB60087	Austria
Thelephora cf. penicillata	EU819494.1	NAMA503	USA
Thelephora ganbajun	EU696946.1	Gb279	China
Thelephora iqbalii	**JX241471**	**MH810**	**Pakistan**
Thelephora palmata	EU819443.1	JMP0085	USA
	AF272919.1	TAA149550	Estonia
Thelephora aff. palmata	AB509755.1	350-421	Japan
Thelephora penicillata	U83484.1	LTT8, Bruns Herbarium, UCB	USA
Thelephora pseudoterrestris	AF272907.1	TAA159625	Estonia
	AB453027.1	CU: Micro: Nan-M11	Thailand
Thelephora regularis	U83485.1	OSC: JMT17371	USA
Thelephora sublilacina	EF493288.1	UP161	Sweden
Thelephora terrestris	HM189959.1	BB23-305-Ah-Pi-150506 (DNA64)	Germany
	HM189958.1	BB64-301-Oh-Pi-150506 (DNA43)	Germany
Thelephoraceae sp. 'Taylor #4'	U83468.1	—	USA
Thelephoroid sp. nw1	AF323110.1	—	USA

Bold indicates sequence generated in this study; all others downloaded from GenBank.

Collection and morphological analysis

During exploration of Himalayan moist temperate forests, an unidentified *Thelephora* specimen associated with *Abies pindrow* was collected near a helipad, in Khanspur, Abbotabad district, Khyber Pakhtunkhwa province, Pakistan. The fresh specimen was morphologically characterized following Corner (1968), and small portions of fresh hymenium were placed into 2% CTAB buffer and stored at –20 °C for future molecular study. The remaining material was dried with a fan heater overnight before storing in Ziploc® bags. Basidiospores, basidia, basidioles and sterigmata were observed in 1% KOH under a compound microscope and measured using an ocular micrometer; drawings were made with the aid of a camera Lucida. Dried specimens are deposited at the Herbarium of the Department of Botany, University of the Punjab, Lahore (LAH).

Molecular analysis

Genomic DNA was extracted with a CTAB method (Gardes & Bruns 1993) modified for silica emulsion binding and purified (Gene-Clean; Q-Biogene). Polymerase chain reactions (PCR) for the ITS-nrDNA region were carried out using the fungus-specific primers ITS1F/ITS4 following protocols described by Gardes & Bruns (1993). PCR products were purified with QIAquick (Qiagen Inc.), and sequenced by Macrogen (South Korea). Maximum Likelihood and Maximum Parsimony methods generated a phylogeny comparing *Thelephora iqbalii* with other thelephoroid species. A total of 22 sequences (21 downloaded from GenBank) were used in the phylogenetic analyses (Table 1), and cladograms were recovered using Mega5. Sequences were aligned with BioEdit version 7.0 (Hall 1999) and ClustalX version 2.0 (Larkin et al. 2007) and corrected manually. All the gaps were treated as 'missing data'. The ITS sequence generated from the holotype was deposited in GenBank.

Results

Thelephora iqbalii Khalid & Hanif, **sp. nov.** Fig. 1

MycoBank MB 800830

Differs from *Thelephora fucoides* by its angular lobate basidiospores and from *T. penicillata* by its coralloid basidiomata and its smaller basidiospores.

Type: Pakistan, Khyber Pakhtunkhwa province, Abbotabad district, Khanaspur, near helipad, 1972 m a.s.l., associated with *Abies pindrow* (Royle ex D. Don) Royle, solitary to gregarious, 22.VIII.2010, M. Hanif, MH810 (**Holotype,** LAH 20810.2; GenBank JX241471).

Etymology: dedicated to Prof. Dr. S.H. Iqbal in honor of his contributions to the knowledge of fungi.

Basidioma 4–5 × 2–4 cm, coralloid, individual branches 1–2 mm thick, long narrow ridges, penicillate with fine hairs on long pointed tips, tip

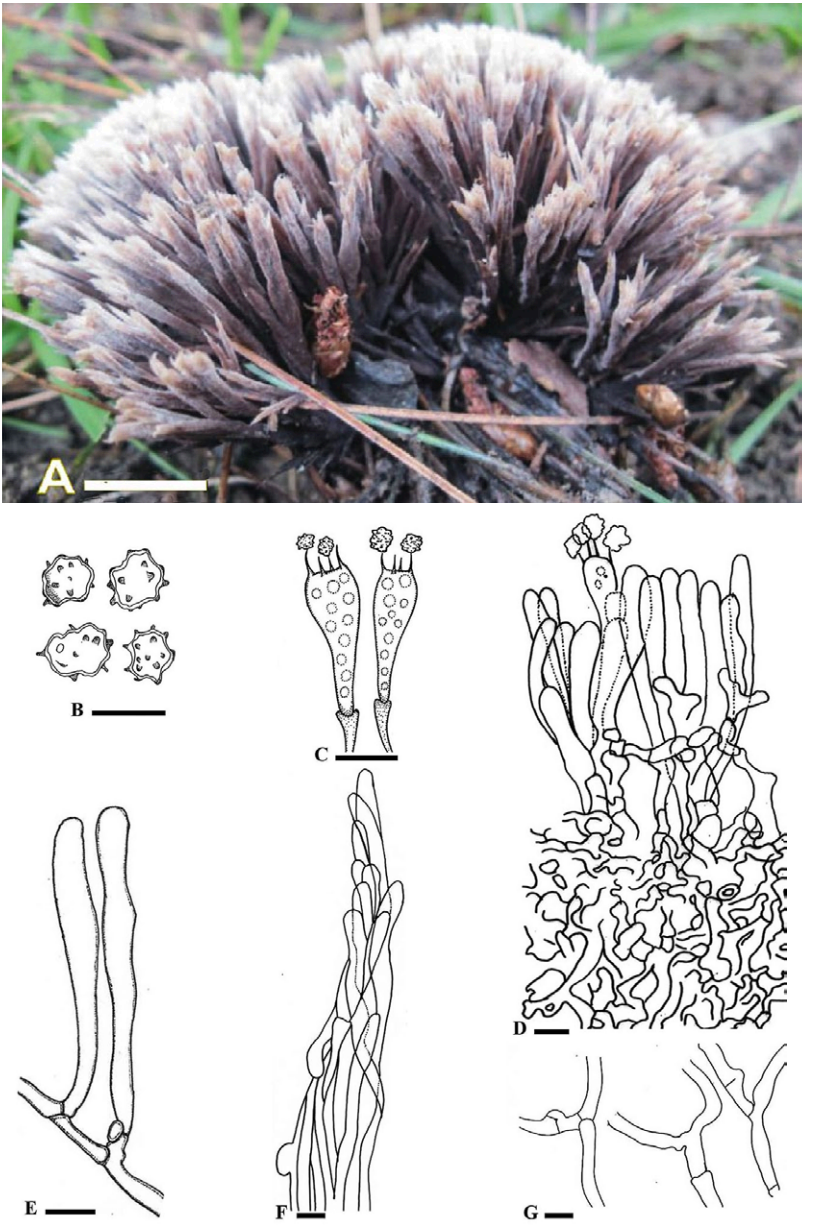

Fig. 1. *Thelephora iqbalii* sp. nov. A. Basidiocarp in situ; B. Basidiospores; C. Basidia; D. Hymenial tissue; E. Basidioles; F. Hair-like appendages of pointed tips; G. Tramal hyphae. Bars: A = 1 cm, B–G = 10 μm.

fibers typically serrate; branches pinkish white at the tip to grayish brown at the base. Context solid, dry.

Hyphal system monomitic, multi-septate, connective hyphae 6 µm diam., skeletal hyphae 5 µm diam., tramal hyphae encrusted with globose to sub-globose crystals, cyanescent in 1% KOH. Basidia 50–75 × 8–10 µm, cylindrical to clavate, with basal clamps, smooth, 2–4 sterigmate, hyaline, guttulate, light blue in KOH. Basidioles 34–40 × 4–8 µm, smooth, thin walled, hyaline, clavate, apex obtuse, light blue in KOH. Basidiospores 6–8.5 × 5–6.5 µm, reniform, angular lobate or scarcely lobate, thick-walled and verrucose, tuberculate ornamentation, brown in lactic acid, light blue in KOH.

Additional material examined ***Thelephora fucoides*: MALAYSIA: Pahang,** Cameron Highlands, 06.08.1934, E.J.H. Corner (E 00192884, holotype).

Phylogenetic analysis

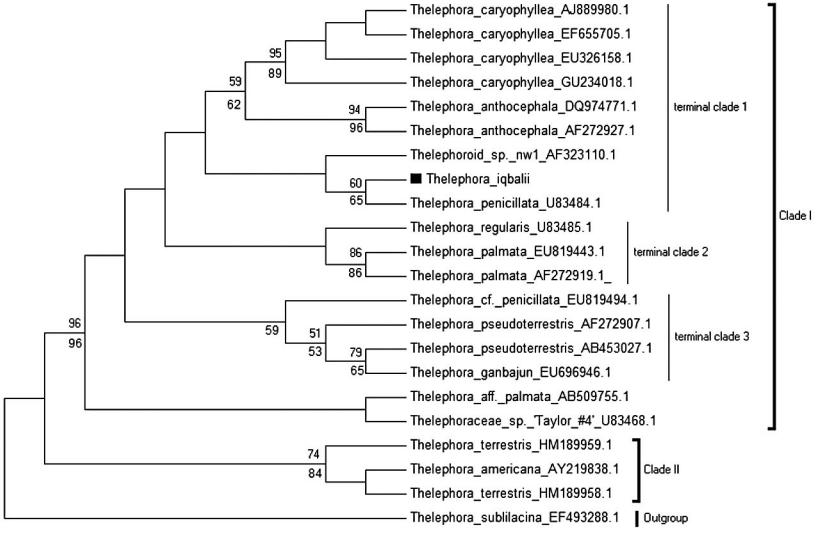

Fig. 2. Cladogram representing phylogenetic analysis inferred from Maximum Parsimony (bootstrap percentages above the lines) and Maximum Likelihood (bootstrap percentages below the lines).

Amplification of the ITS-nrDNA region produced one sequence of 654 bp. Initial BLAST analysis showed a 91% maximum identity with *Thelephora penicillata* (GenBank U83484) with 100% query coverage.

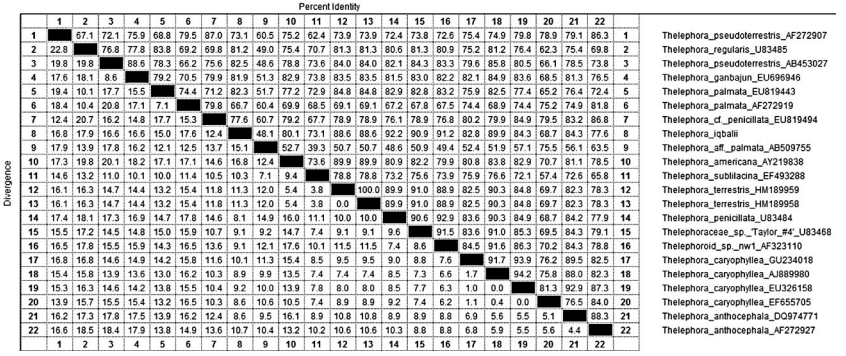

Fig. 3. Percent genetic divergence and percent similarity for *Thelephora* spp.

After trimming 84 unaligned characters from the 3′ and 5′ ends of the 789-bp ITS region, 705 characters were used for final analysis, of which 360 were conserved, 301 were variable but parsimony uninformative, 172 were parsimony informative, and 133 were singletons. The phylogenetic tree generated two major clades (Fig. 2). *Thelephora iqbalii* clustered with *T. penicillata* within terminal clade I of clade I, and *T. anthocephala* and *T. caryophyllea* appeared as sister species to *T. iqbalii*. *Thelephora iqbalii* and *T. penicillata* shared 91.8% genetic characters/nucleotides and showed an 8.1% genetic divergence (Fig. 3). However, we should note that the clustering of all these species in terminal clade I of clade I (Fig. 2) was not supported by both analyses.

Discussion

Eight *Thelephora* species have been previously recorded from Pakistan (Ahmad 1956, 1972). Our new species, *T. iqbalii,* which is supported both by morphological and ITS sequence analysis, shares the basic morphological characters with other *Thelephora* species: spiny to warty and often lobed basidiospores, drab basidiocarp coloration, and blue to green KOH reactions (Corner 1968). Hanif (2012) reported a rich diversity of *Thelephorales* in subterranean ectomycorrhizal communities in HMT forests, and *T. iqbalii* is expected to exhibit a similar ectomycorrhizal association typical of *Thelephora* species elsewhere in Pakistan.

In Ahmad's (1972) key to *Thelephora* species in Pakistan, *T. iqbalii* would fall close to *T. fucoides,* which differs by its echinulate basidiospore ornamentation (Corner 1968). Morphological and phylogenetic analyses

imply a close alliance between *T. penicillata* and *T. iqbalii*. However *T. penicillata* can be distinguished by its resupinate basidiomes with prostrate to suberect branches, its smaller basidiomata with margins that are cristate, penicillate ramose-spiculose, or fimbriate, and its slightly larger basidiospores (Corner 1968).

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